Amendments to the Specification

Please replace the paragraph beginning at page 4, line 24, with the following rewritten paragraph:

--Figures 6A, B, and C are 2-D or 1-D gel comparisons of the proteins in microdissected normal and tumor epithelium. Fifty thousand cells were procured by LCM, directly lysed in IEF buffer and run on a 3-10 NL Pharmacia IPG IEF strip for 100kVhr. The second dimension runs were performed on 8-18% linear gradient SDS-PAGE gels and the gels were stained with silver. Figures 6A and 6B show match tumor and normal ~~n rmal~~ fingerprints for each patient. A representative pI and molecular weight ruler for direct comparison and alignment is shown in panel A. Figure 6C shows the alpha-tubulin immunoblot that was used to normalize for relative protein load.--

Please replace the paragraph beginning at page 5, line 23, with the following rewritten paragraph:

--Figures 13A, B, and C show data which indicates SELDI protein profiles of LCM-derived cellular lysates are reproducible and sensitive. Figure 13A shows two separate microdissections of prostate tumor epithelium from ~~form~~ the same tissue section from the same patient (1200[)] cells each) which were analyzed by SELDI protein fingerprinting. The raw mass spectroscopic mass map is shown for each microdissection along with the Gel-View® display from the same data set. Figure 13B shows two separate microdissections of prostate tumor epithelium from ~~fr m~~ a tissue section from two different patients (1200 cells each) which were analyzed by SELDI protein fingerprinting. The raw mass spectroscopic mass map is shown for each microdissection along with the Gel-View® display from the same data set. Figure 13C shows four separate microdissections of decreasing number of cells that were analyzed by SELDI protein fingerprinting. The Gel-View® display is shown as a representation of the direct alignment of each of these four mass spectra to each other.--

Please replace the paragraph beginning on page 6, line 19, with the following rewritten paragraph:

--Figures 17A and 17B are SELDI analyses of microdissected esophageal epithelium showing proteins disregulated in a disease-specific manner. Figures 18A and 18B are SELDI analyses of 8 different esophageal cancer cases, where three separate microdissections of eight different patients' matched tumor and normal cells were subjected to SELDI analysis via the use of a hydrophobic interaction C18 binding surface. Each replicate was run in triplicate, giving a total of 72 data points for

each protein peak analyzed. The analysis of the protein fingerprint in the low mass region is shown in Figure 17A, the higher mass region in Figure 17B. A representative mass map from one case (case #1) is shown on the left side of each panel with the normal and tumor fingerprint shown (top and bottom, respectively) for each mass region. A gel-like representation is displayed for that particular case as well as the fingerprint for two other cases. Proteins 1, 2, 6, and 7 are labeled for orientation. All cases analyzed in the study set were then subjected to analysis as a ration of relative intensity of the selected proteins to one another and the statistical results shown ~~sonw~~ on the right side of each figure. Figure 17A: average C.V. = 12.7%, 29.4% for the normal and tumor microdissections respectively. Figure 17B: average C.V. = 10.5% and 18.9% for the normal and tumor microdissections, respectively.--

Please replace the paragraph beginning at page 7, line 27, with the following rewritten paragraph:

--Figures 21A, 21B, and 21C diagram the reproducibility of [f] protein analysis for samples microdissected from a mixed sample of epithelial cells of the esophagus. All the following data was obtained from normal cells present in the sample. Figure 21A shows the reproducibility of the annexin I protein data in normal cells over a variation of "shot" size within one slide and between multiple slide sets. Figure 21B shows a histogram of the coefficient of variance for these data sets. Figure 21C graphically shows reproducibility of the total protein obtained from the cells as shown by fluorescence detection.--

Please replace the paragraph beginning at page 12, line 1, with the following rewritten paragraph:

--A further analysis that may be performed ~~perf rmed~~ involves the use of the surface enhanced laser desorption ionization spectroscopy technique, or SELDI (Ciphergen Biosystems Inc., Palo Alto, CA). This process can separate proteins which would not be separately focused by 2-D gel analysis, in particular those proteins which are very basic, very small (<7000 daltons) or are expressed at low or moderate levels in the cells. The lower level of expression becomes critical in these experiments because of the extremely small sample size of cells used. SELDI also separates proteins more rapidly than gel analysis. SELDI utilizes a "protein chip" that allows for desorption and detection of intact proteins at the femtomole levels from crude samples. Proteins of interest are directly applied to a defined small surface area of the protein chip formatted in 8 to 24 predetermined regions on an aluminum support. These surfaces are coated with defined chemical "bait" matrices comprised of standard chromatographic supports, such as hydrophobic, cationic, or anionic or biochemical bait molecules such as purified protein ligands, receptors, antibodies, or DNA oligonucleotides. See Strauss, New ways to probe the molecules

of life, 282 Science 1406 (1998). In the case of LCM collected samples, the solubilized proteins are applied to the surface of the SELDI chip. Binding of the proteins to the surface is dependent on the nature of the bait surface and the wash conditions employed. The mixture of bound proteins is then characterized by laser desorption and ionization and subsequent time of flight mass analysis generated from a sensitive molecular weight detector. This data produces a protein fingerprint for the sample, with SELDI having a practical resolution and detection working range of 1000 to 300,000 daltons, depending on the energy absorbing molecule utilized and the bait surface/wash conditions employed.--

Please replace the paragraph header at page 15, line 1, with the following rewritten paragraph header:

--Extraction~~Extracti n~~ Buffer--

Please replace the paragraph beginning at page 17, line 10, with the following rewritten paragraph:

--The present technology provides one of the first direct estimates of the actual number of protein molecules per tissue cell *in vivo* for a single specific known protein of moderate to low abundance. The number of total PSA molecules in normal prostate epithelium ranged from 10^4 to 10^6. PSA is an important serum analyte used to clinically monitor prostate cancer, but it is not a specific maker of prostate cancer. Previous investigators have reported great heterogeneity in the intensity of PSA immunohistochemical staining among various neoplastic and non-neoplastic populations in the prostate. Populations of microdissected cells of a pure histologic class would be expected to contain some level of heterogeneity in PSA expression among the population members, as presently seen.--

Please replace the paragraph header at page 23, line 1, with the following rewritten paragraph header:

--Protein ~~Confirmati n~~Confirmation – Immunoblot--

Please replace the paragraph header at page 24, line 1, with the following rewritten paragraph header:

~~--Validati n~~Validation and Extension [t] to Other Cancers--

Please replace the paragraph beginning at page 29, line 6, with the following rewritten paragraph:

--Frozen tissue was obtained from radical prostatectomy specimens and embedded in OCT compound (Tissue-Tek, Miles, Elkhart, IN). Eight micron sections were made with a standard cryostat and stained with hemotoxylin and eosin using standard protocols. Benign and malignant histology was identified by a pathologist and LCM was performed to obtain cells from each population by directing the laser at those populations of cells. LCM was performed ~~perfomred~~ as previously described, except AEBSF (Boeringer Manheim) was added to the staining baths at a final concentration of 2 mM to inhibit proteases. For 1-dimensional and 2-dimensional electrophoresis analysis 2,000 (approximately 8 - 10,000 cells) and 5,000 (approximately 20 - 25,000 cells) 30 micron laser shots of each cell population were used, respectively. Based on careful review of histologic sections each dissection is estimated to contain > 95% of desired cells.--

Please replace the paragraph header at page 31, line 26, with the following rewritten paragraph header:

-- ~~Producti n~~Production of SELDI Protein Fingerprints ~~f r~~for ~~Tum r~~Tumor and Nontumor Cell Samples --

Please replace the paragraph header at page 34, line 1, with the following rewritten paragraph header:

--Discriminatory ~~Diseriminat ry~~ SELDI analysis of] f] different tumor types—

Please replace the paragraph beginning at page 36, line 27, with the following rewritten paragraph:

--To apply the present methods to this type of analysis, the tissue from multiple patients is exposed to the agent to be tested. The exposure can be done *in vivo*, prior to collection of the sample, or *in vitro*, after collection of the sample and/or after laser microdissection. *In vivo* exposure would involve administration of the agent to the subject, while *in vitro* administration could be administration ~~adminsitration~~ to cultured cells. Some agents that could be tested include pharmacological agents, imaging agents, labeled proteins, such as ligands, or other agents known to have particular effects on cells, such as cytokines. After exposure, microdissection techniques are used to isolate the cells of interest from the sample, the cells are lysed to allow isolation of the proteins or other cellular components, such as nucleic acids or other subcellular structures, from the sample, and the lysate contents are transferred to a confined zone of a substrate. The lystate contents, or cellular components, are placed in

identifiable positions on a substrate, where such positions are confined zones. One example of a confined zone is the coordinates of an array. The array is constructed by applying microspots of the isolated proteins on any suitable matrix, such as nitrocellulose, nylon, or silica.--

Please replace the paragraph beginning at page 38, line 23, with the following rewritten paragraph:

--An additional application of tissue array technology to the methods of the present invention is the use of the protein content assay to prescreen various tissue types for the target of diagnostic or therapeutic moieties. In this embodiment, a therapeutic or diagnostic agent is being evaluated for its appropriateness ~~appr priateness~~ in a patient's treatment. If the target of this agent is known, the presence or absence of this target in various cell types of the patient can be tested. Thus, using the breast tissue of Figure 19 as an example, if it was questionable whether a target enzyme for a chemotherapeutic agent was present in a particular patient's cancerous cells, the samples are generated and placed in an array as illustrated. Then an antibody or other means of identification of the target is used to determine if the target agent is present. This allows diagnostic, imaging, or therapeutic agents to be specifically selected for patients or disease types based on the presence of the target molecule in the diseased tissue.--

Please replace the paragraph beginning at page 39, line 27, with the following rewritten paragraph:

--This plate is positioned on[n] the stage of Genetic Micro Systems 417 Arrayer. Oncyte Slides (Schleicher & Schuell) covered in pure nitrocellulose are placed in the appropriate positions on the arrayer. After the desired number of slides and replicates has been programmed into the accompanying software, the instrument is activated. A metal micro tube lowers into the appropriate well and lifts 1 µl of the lysate, trapping it inside the bore of the tube. After positioning the tube over the nitrocellulose slide, a pin pierces the trapped lysate, spotting a minute aliquot onto the nitrocellulose surface measuring 500 µm in diameter.--